Acta Botanica Brasilica
doi: 10.1590/0102-33062018abb0174

Fruit of *Lepidaploa* (Cass.) Cass. (Vernonieae, Asteraceae): anatomy and taxonomic implications

Danilo Marques[1*], Rafael de Oliveira Franca[2], Massimiliano Dematteis[1] and Juliana Marzinek[3]

Received: May 8, 2018
Accepted: June 5, 2018

ABSTRACT

Lepidaploa is a taxonomically complex genus of Vernonieae, which is difficult to delimit morphologically due to vegetative and reproductive characters that overlap with *Chrysolaena* and *Lessingianthus*. Anatomical studies of cypselae are taxonomically useful for delimiting subtribes, genera and species of Asteraceae, and especially within Astereae and Eupatorieae. Given the importance of cypselae to the taxonomy of tribes of Asteraceae, we searched for morphological patterns among the species of *Lepidaploa*. Using light microscopy, we analyzed fruits of 21 species of *Lepidaploa* to evaluate the taxonomic position of the genus and species of questionable placement in the group. Our results showed that the morphologies of the cypselae of species of *Lepidaploa* are homogeneous and similar to other species of Vernonieae. However, pappus vascularization and the number of mesocarp layers could be useful for differentiating the sister groups *Chrysolaena*, *Lepidaploa* and *Lessingianthus*, which present similar macro- and micro-morphological, palynological and chromosomal characters. Also, the presence of glandular trichomes and idioblasts in the cypsela, and lignified cells in the carpopodium exocarp, can be used to separate closely related species.

Keywords: carpopodium, Compositae, cypsela, floral disk, pappus, *Vernonia*

Introduction

Vernonieae is one of the most species-rich tribes of the Compositae, comprising about 1500 species distributed in Africa, Asia and the Americas (Keeley & Robinson 2009). Although Vernonieae exhibits a high degree of morphological variability, the tribe is generally known for its alternate leaves, discoid heads with violet flowers, acute style branches and biseriate pappus (Keeley & Robinson 2009). The cypselae of Vernonieae are generally cylindrical or turbinate, sometimes prismatic, and brown (Semir *et al*. 2011; Marques & Dematteis 2014; Angulo *et al*. 2015; Talukdar 2015; Redonda-Martínez *et al*. 2017; Pico *et al*. 2016). The pappus can be biseriate with an outer short paleaceous series and an inner long bristle series (Marques & Dematteis 2014; Angulo *et al*. 2015; Redonda-Martínez *et al*. 2017; Pico *et al*. 2016), biseriate with two paleaceous series (one short and one long) (Semir *et al*. 2011), or it can be absent (Redonda-Martínez *et al*. 2017).

Due to the taxonomic importance of cypselae for the delimitation of subtribes, genera and species of Vernonieae, numerous anatomical studies have been performed with taxa of this tribe (Misra 1972; Pandey & Singh 1980; Mukherjee & Sarkar 2001; Martins & Oliveira 2007; Galastri & Oliveira 2010; Jana & Mukherjee 2013; Freitas *et al*. 2015; Talukdar 2015; Redonda-Martínez *et al*. 2017). Most of the African and Asian species of Vernonieae that have been studied anatomically have revealed that the anatomy of the cypselae is very important to the taxonomy valuable

1 Instituto de Botánica del Nordeste, Universidad Nacional del Nordeste-Consejo Nacional de Investigaciones Científicas y Técnicas, W3400BCH, Corrientes, Argentina
2 Programa de Pós-graduação em Biologia Comparada, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil
3 Programa de Pós-graduação em Biologia Vegetal, Instituto de Biologia, Universidade Federal de Uberlândia, 38400-902, Uberlândia, MG, Brazil

* Corresponding author: danilobioufu@gmail.com

of the tribe (Mukherjee & Sarkar 2001; Jana & Mukherjee 2013; Talukdar 2015). Some American species have also been studied, and their anatomical characters have also proven to be important to the taxonomy of the group (Martins & Oliveira 2007; Galastri & Oliveira 2010; Freitas *et al*. 2015; Redonda-Martínez *et al*. 2017).

Vernonieae is also known for the uncertain taxonomic delimitations of its contained taxa (Funk *et al.* 2005; Keeley *et al.* 2007). Part of the difficulty in defining generic limits for this tribe has been due to the fact that *Vernonia s.l.* (ca. 1000 spp.) encompasses a large number of species with many different vegetative and reproductive features (Keeley & Jansen 1994; Keeley *et al.* 2007). Robinson (1999) compiled previous studies on the genus and delimited several other genera from *Vernonia s.l.* based on palynological, macro- and micromorphological, and cytological data.

Chrysolaena, *Lepidaploa* and *Lessingianthus* are three closely related genera that were thus segregated from *Vernonia* s. l. (Robinson 1999; Keeley & Robinson 2009). However, the delimitation of these three genera from one another is still complex due to the superposition of macro- and micromorphological characters, such as the type of inflorescence, shape of the heads, number of florets, absence or presence of a basal node, and indument of florets and cypselae, for example. Martins & Oliveira (2007) and Galastri & Oliveira (2010) have been the only studies to provide anatomical data on *Chrysolaena* and *Lessingianthus*, while such studies with *Lepidaploa* are practically non-existent, except for anatomical studies o the cypsela of *Lepidaploa tortuosa* (Redonda-Martínez *et al.* 2017).

The genus *Lepidaploa* contains about 150 species that occur in the Neotropics (Pruski 2017), with Brazil and Bolivia being the countries with the highest species richness (Redonda-Martínez & Villaseñor 2011). Some species of *Lepidaploa* that inhabit southern South America (Argentina, Bolivia, Paraguay and southern Brazil) are of uncertain taxonomic position due to the overlap of vegetative and reproductive characters among species.

Given the limited anatomical knowledge about American Vernonieae, we studied the anatomy of the cypselae of some species of *Lepidaploa* that inhabit southern South America. Therefore, this study describes and compares the anatomy of the cypselae of species of *Lepidaploa* with the aim of identifying morphological patterns for the genus and its included species.

Materials and methods

A total of 21 species of *Lepidaploa* (Cass.) Cass. from southern South America were sampled. Details, including voucher information, are provided in Table 1.

For anatomical analysis, dried mature and immature cypselae were rehydrated with 5 N NaOH solution (Anderson 1963, modified from 36 to 4 hours), dehydrated in an ethanol series and embedded in historesin (Leica Microsystems, Heidelberg, Germany). The samples were sectioned using a rotary microtome at 10 µm thickness. The material was then stained with 0.05 % toluidine blue in acetate buffer, pH 4.7 (O'Brien *et al.* 1964 modified) and mounted with synthetic resin. The slides were analyzed under a light microscope (Olympus BX41) and digital images acquired. For images of the pericarp, we used sections of the median region of the cypselae.

Results

All of the species analyzed had a prismatic cypsela in cross section (Figs. 1-6). The ribs along the fruit can be distinct (Figs. 1A, C, E, G; 2A, C, E, G; 3A, C, E, G; 4A, C, E, G; 5A, C, E; 6A, D, F) or indistinct, as in some regions of the cypsela of *L. amambaia* (Fig. 1A), *L. psilostachya* (Fig. 5A), and *L. sordidopapposa* (Fig. 6D) (Tab. 2). The fruit comprises exocarp and mesocarp; the endocarp is only intact in *L. balansae* (Fig. 1H) and *L. setososquamosa* (Tab. 2).

Exocarp: All species possess a uniseriate exocarp with periclinally elongated cells (Figs. 1B, D, F, H; 2B, D, F, H; 3B, D, F, H; 4B, D, F, H; 5B, D, F; 6B, E, G). Generally, the cells of the exocarp are smaller in the ribs of *L. amambaia* (Fig. 1B), *L. argyrotricha* (Fig. 1D), *L. balansae* (Fig. 1H), *L. buchtienii* (Fig. 2D), *L. canescens* (Fig. 2F), *L. chamissonis* (Fig. 2H), *L. costata* (Fig. 3B), *L. eriolepis* (Fig. 3F), *L. mapirensis*, *L. myriocephala* (Fig. 4B), *L. psilostachya*, *L. remotiflora* (Fig. 5D), *L. salzmannii* (Fig. 5F), *L. setososquamosa*, and *L. tarijensis* (see Tab. 2). All species have biseriate non-glandular trichomes (Figs. 1C, F; 2B, D, F; 3B, D, F; 4F; 5F; 6B, E) while only *L. chamissonis*, *L. pseudomuricata*, *L. salzmannii*, and *L. setososquamosa* (Fig. 6C) possess glandular trichomes. Stomata were observed among the cells of the exocarp only in *L. setososquamosa* (Fig. 7A). Cells with secretory appearance (idioblasts) are found in the exocarp of most species. Compared with adjacent cells, idioblasts are larger, spherical or, in a few cases, ovate-spherical, and have dense content (Figs. 1D, F, H; 2B, D, H; 3B, D; 4D, F, H; 5F; 6E, G; 7 I). These cells are absent in *L. amambaia* (Fig. 1B), *L. canescens* (Fig. 2F), *L. eriolepis* (Fig. 3F), *L. mapirensis* (Fig. 3H), and *L. myriocephala* (Fig. 4B) (Tab. 2).

Mesocarp: This region comprises one to nine layers of cells (Figs. 1B, D, F, H; 2B, D, F, H; 3B, D, F, H; 4B, D, F, H; 5B, D, F; 6B, E, G). Two distinct regions are observed in the mesocarp. The outer mesocarp is formed, generally, of one or two layers of cells, except in *L. chamissonis* (Fig. 2G, H), *L. myriocephala*, and *L. remotiflora* (Fig. 5C, D), which, in some regions, can have more than two layers of cells. The cells of the outer mesocarp can be periclinally elongated or isodiametric. They are small compared to the cells of the inner mesocarp (i.e., *L. balansae* - Fig. 1H). The wall of the mesocarp cells can be formed of primary or secondary walls. Crystals are observed in the outer mesocarp of all species, mainly in the cell layer closest to the exocarp (Figs. 1D; 4D, F, H; 5B, D, F) (Tab. 2). In most of the species, the

Table 1. Voucher information for species of *Lepidaploa* used in the present study.

Species	Voucher	Country and Locality
L. amambaia H. Rob.	M. Dematteis & A. Schinini 867 (CTES)	Paraguay. Amambay, 25 km of N de J. P. Caballero.
	M. Dematteis *et al.* 3336 (CTES)	Paraguay. Concepción, 25 km W de San Carlos del Apa. Estancia Arrecifes.
L. argyrotricha (Sch. Bip. ex Baker) H.Rob.	E. Barbosa *et al.*1012 (CTES)	Brazil. Paraná, Bocaiuva do Sul.
L. bakerana (Britton) H. Rob.	A. Fuentes *et al.* 7042 (CTES)	Bolivia. La Paz, Province Muñecas.
	St. G. Beck 8317 (CTES)	Bolivia. La Paz, Province Nor Yungas.
L. balansae (Hieron.) H. Rob.	E. Adenesky s.n. (FLOR 4542)	Brasil. Santa Catarina, Chapecó.
	E. Valduga & E. Pasini 81 (FURB)	Brasil. Rio Grande do Sul, Bento Gonçalves-Vale dos Vinhedos.
	J.A. Jarenkow 1689 (FLOR)	Brasil. Rio Grande do Sul, Lajeado, Vale do Arroio.
L. beckii H. Rob.	St. G. Beck 21420 (CTES)	Bolivia. La Paz, Province Nor Yungas, Coroico.
	St. G. Beck 14931 (CTES)	Bolivia. La Paz, Province Nor Yungas.
L. buchtienii (Gleason) H. Rob.	St. G. Beck 29463 (CTES)	Bolivia. La Paz, Provincia Larecaja.
L. canescens (Kunth) H. Rob.	St. G. Beck 12108 (CTES)	Bolivia. La Paz, Sud Yuangas, Chulumani.
L. chamissonis (Less.) H. Rob.	L. A. Funez 1928 (FURB)	Brasil. Santa Catarina, Itajaí.
	M. Verdi *et al.* 4028 (FURB)	Brasil. Santa Catarina, Imaruí, Parque Estadual da Serra do Tabuleiro.
L. costata (Rusby) H. Rob.	St. G. Beck 29455 (CTES)	Bolivia. La Paz, Province Larecaja.
	St. G. Beck 28282 (CTES)	Bolivia. La Paz, Province Larecaja, Villaque Poroma, Vale del rio Sapucuni.
	J.R.I. Wood & D. Wasshausen 13906 (CTES)	Bolivia. La Paz, Larecajaca, 25-30 km N of Caravanito.
L. deflexa (Rusby) H. Rob.	St. G. Beck 29174 (CTES)	Bolivia. La Paz, Province J. Bautista Saavedra, Apolo, Yurilaya.
	St. G. Beck 22758 (CTES)	Bolivia. Departamento La Paz, Province Sud Yungas.
L. eriolepis (Gardner) H. Rob.	A. Stival-Santos *et al.* 785 (FURB)	Brasil. Santa Catarina, Santo Amaro da Imperatriz, Cova da Onça.
	A. Korte & A. Kniess 4010 (FURB)	Brasil. Santa Catarina, Nova Trento, Valsugana.
L. mapirensis (Gleason) H. Rob.	J.C. Solomon 9656 (CTES)	Bolivia. La Paz, Province Nor Yungas, 0.9 km W of Chuspipata.
	J.C. Solomon 15318 (CTES)	Bolivia. La Paz. Province Nor Yungas.
L. myriocephala (DC.) H. Rob.	M. Dematteis 1221 (CTES)	Bolivia. La Paz, Province Larecaja.
	M. Dematteis 1116 (CTES)	Bolivia. La Paz, Provincia Nor Yungas, 2 km N of Canavari.
L. novarae (Cabrera)	O. Ahumada & J. Aguero 8365 (CTES)	Argentina. Salta, Santa Victoria, Parque San Martín.
	Vervoorst 4581 (CTES)	Argentina. Salta, Santa Victoria, Parque San Martín.
L. pseudomuricata H. Rob.	M. Verdi *et al.* 5909 (FURB)	Brasil. Santa Catarina, Bom Jardim da Serra, Farm Papagaios.
	A. Korte *et al.* 5917 (FURB)	Brasil. Santa Catarina, Campo Alegre, Campinas dos Faxinais.
	M. Verdi *et al.* 5874 (FURB)	Brasil. Santa Catarina, Pouso Redondo, Corruchel.
L. psilostachya (DC.) H. Rob.	A. Krapovickas & C.L. Cristóbal 44612 (CTES)	Argentina. Misiones, San Ignacio, Quiroga's house.
	E. Cabral *et al.* 348 (CTES)	Argentina. Misiones, San Ignacio.
L. remotiflora (Rich.) H. Rob.	H. A. Keller 4096 (CTES)	Argentina. Misiones, General San Martín, Route 211.
	H. A. Keller 6416 (CTES)	Argentina. Misiones, San Ignacio, Club del Río.
	E. Guitierrez & E. Chavez 2185 (CTES)	Bolivia, German Busch., Rio Negro.
L. salzmannii (DC.) H. Rob.	M. Dematteis 1020 (CTES)	Bolivia. Santa Cruz, Ichilo, 4 km E of San Carlos.
	M. Dematteis 3438 (CTES)	Bolivia. Tarija, O'Connor, 8,3 km E of Entre Rios.
L. setososquamosa (Hieron.) M.B. Angulo & M. Dematt.	L. J. Novara 10877 (CTES)	Argentina. Salta, Rosario de Lerma, Dique Las Lomitas.
	V. S. Neffa *et al.* 1305 (CTES)	Bolivia. Santa Cruz. Chiquitos, 15 km E of Ipias.
	A. Krapovickas & C. L. Cristóbal 44197 (CTES)	Paraguay. Boquerón, Parque Valle Natural, 25 km S of Filadelfia.
L. sordidopapposa (Hieron.) H. Rob.	St. G. Beck 22672 (CTES)	Bolivia. La Paz, Province Nor Yungas.
	St. G. Beck 17717 (CTES)	Bolivia. La Paz, Province Nor Yungas.
L. tarijensis (Griseb.) H. Rob.	A. Krapovickas *et al.* 19425 (CTES)	Argentina. Salta, General San Martín, Pocitos.
	A. G. Schinini 5189 (CTES)	Argentina. Salta, Quebrada de la Virgen.

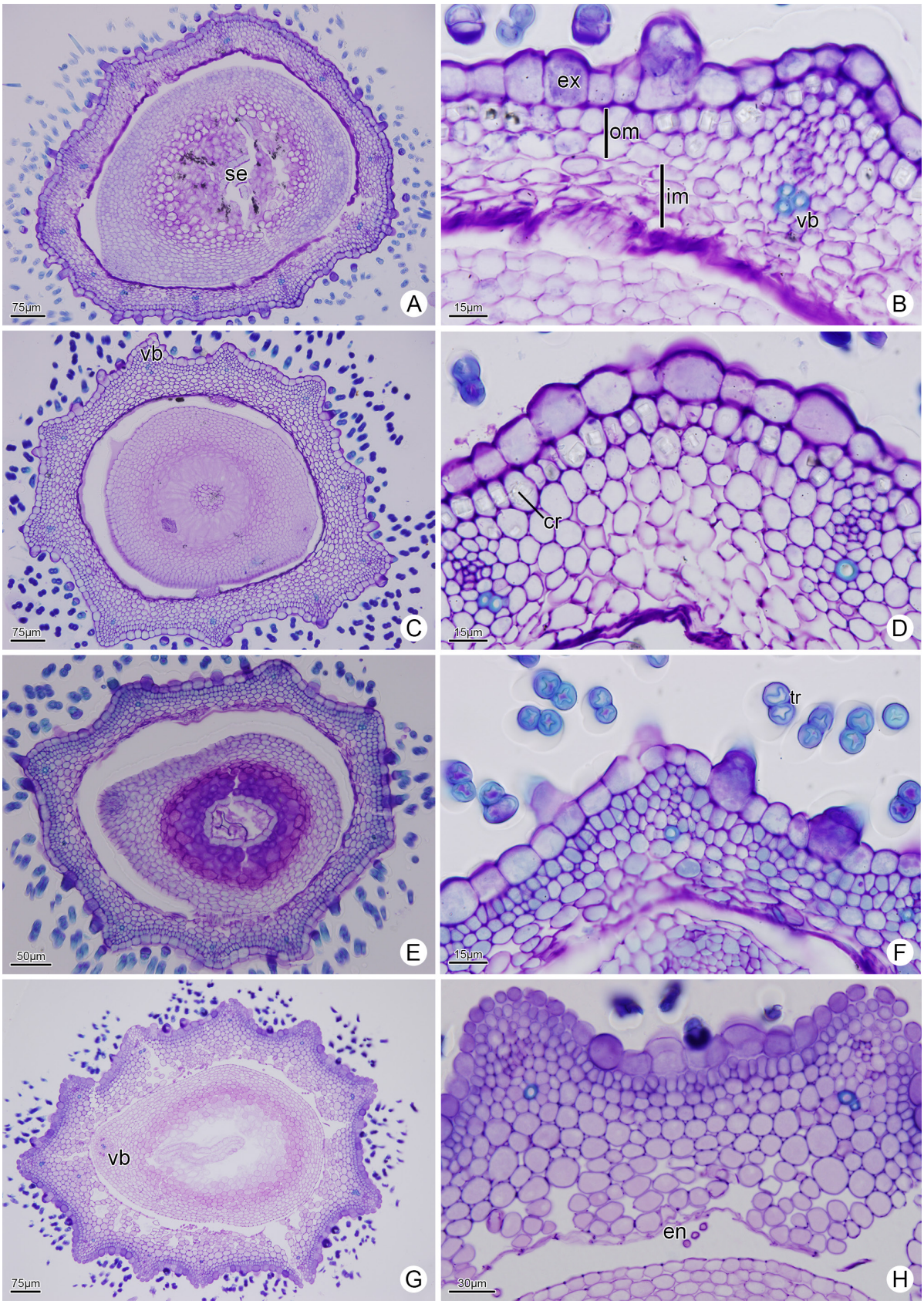

Figure 1. Transversal sections of the fruit of the studied species of *Lepidaploa*: **A-B.** *L. amambaia*; **C-D.** *L. argyrotricha*, in D detail of crystals in the outer mesocarp; **E-F.** *L. bakerana*; **G-H.** *L. balansae*, in H note the endocarp. cr: crystals; ex: exocarp; im: inner mesocarp; om: outer mesocarp; se: seed; tr: trichome; vb: vascular bundles; arrowhead: vascular bundles in seed.

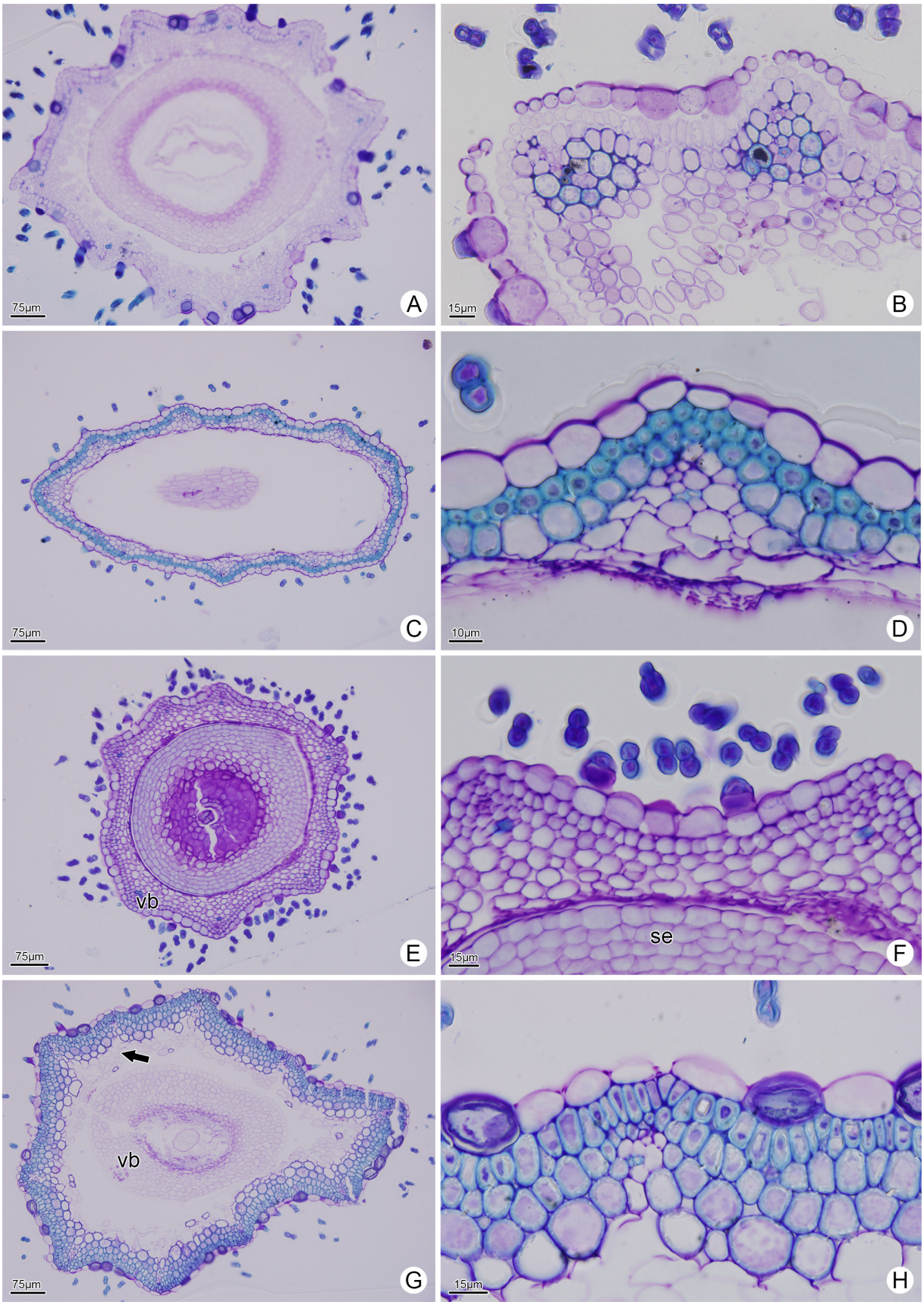

Figure 2. Transversal sections of the fruit of the studied species of *Lepidaploa*: **A-B.** *L. beckii*; **C-D.** *L. buchtienii*; **E-F.** *L. canescens*; **G-H.** *L. chamissonis*; arrow: disintegrating inner mesocarp; se: seed; vb: vascular bundles.

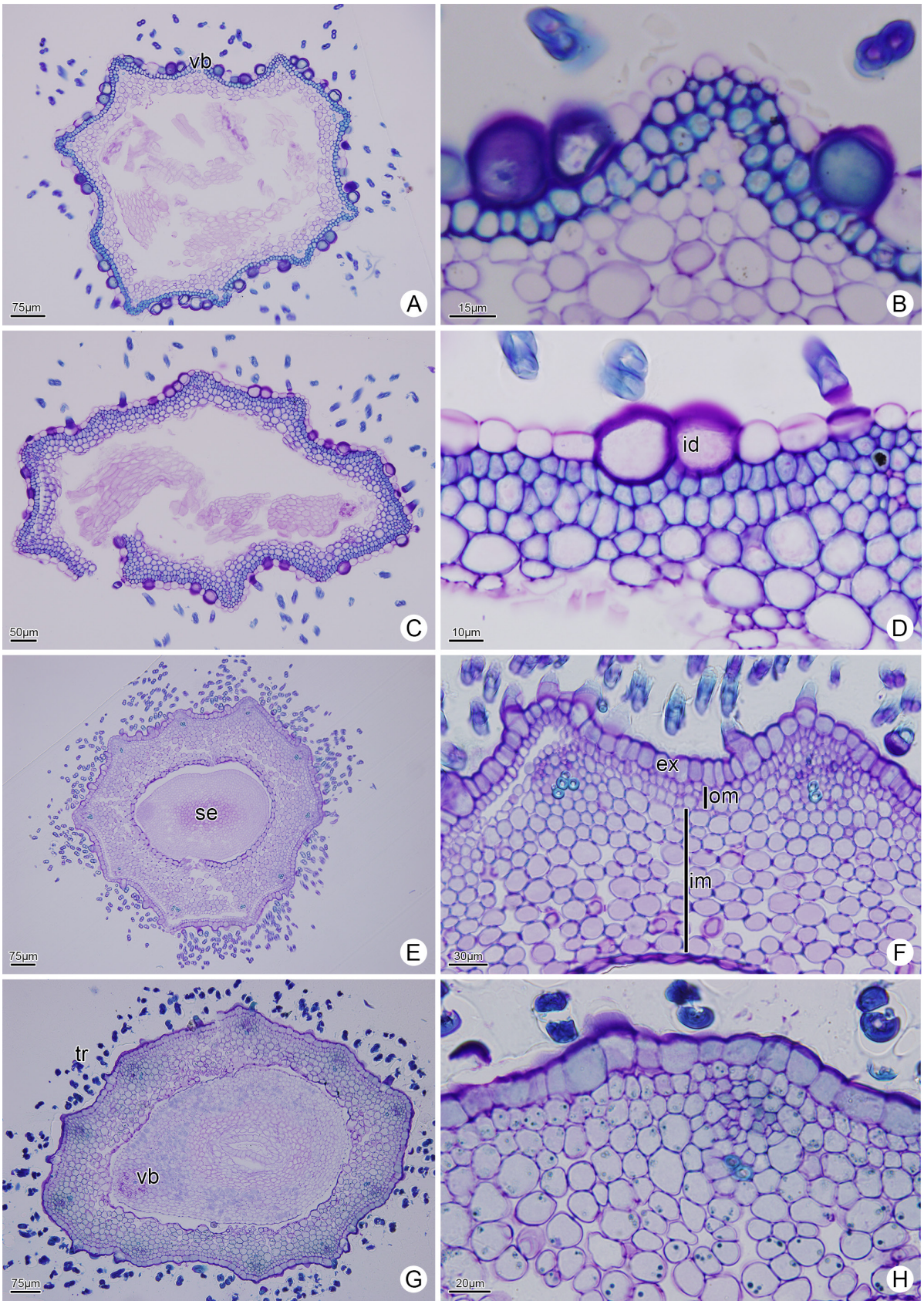

Figure 3. Transversal sections of the fruit of the studied species of *Lepidaploa* : **A-B.** *L. costata*; **C-D.** *L. deflexa*, in D detail of idioblasts in the exocarp. **E-F.** *L. eriolepis*; **G-H.** *L. mapirensis*. ex: exocarp; id: idioblasts; im: inner mesocarp; om: outer mesocarp; se: seed; tr: trichome; vb: vascular bundles.

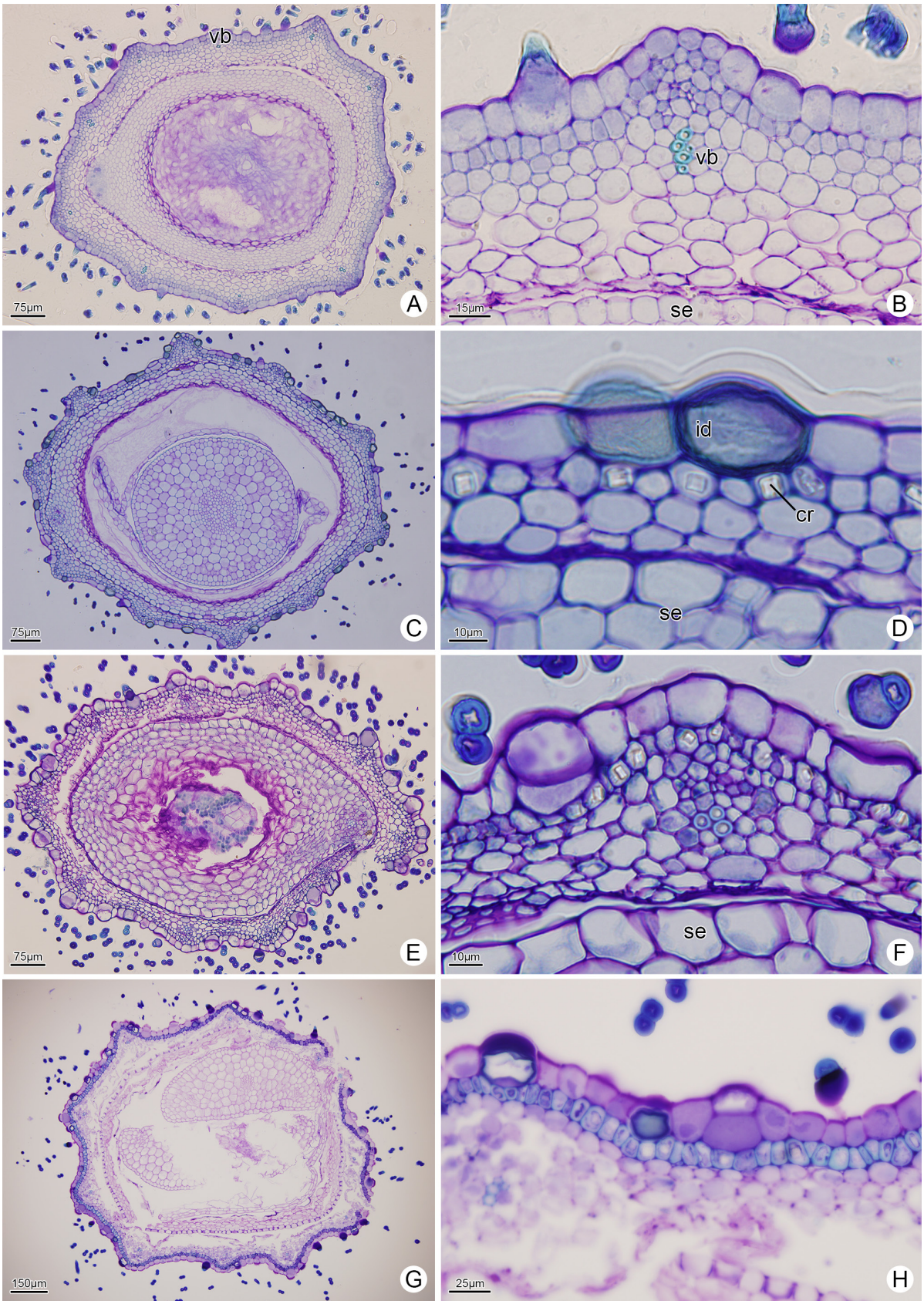

Figure 4. Transversal sections of the fruit of the studied species of *Lepidaploa*: **A-B.** *L. myriocephala*; **C-D.** *L. novarae*, in D detail of idioblasts in the exocarp and detail of the crystals in the outer mesocarp; **E-F.** Immature cypsela of *L. pseudomuricata*; **G-H.** Mature cypsela of *L. pseudomuricata*. cr: crystals; id: idioblasts; se: seed; vb: vascular bundles.

inner mesocarp is partially disintegrated (Figs. 2G; 5C) and comprises two to seven layers of cells (Figs. 1B, D, F, H; 2B, D, F, H; 3B, D, F, H; 4B, D, F, H; 5B, D, F; 6B, E, G). However, the inner mesocarp is wholly consumed in mature cypselae of *L. psilostachya* (Fig. 5B). The cells of the inner mesocarp are parenchymatic with some intercellular spaces (Figs. 1B, D, F, H; 2B, D, F, H; 3B, D, F, H; 4B, D, F, H; 5B, D, F; 6B, E, G). Collateral vascular bundles are present in the mesocarp of all species (Figs. 1C; 2E; 3A; 4A; 5E; 6A). The majority of cypselae possess ten vascular bundles per cypsela, but in some species the number of vascular bundles is variable. For example, some samples of *L. amambaia* (Fig. 1A), *L. argyrotricha* and *L. beckii* (Fig. 2A) had 11 vascular bundles per cypsela.

Endocarp: The endocarp is consumed in the middle region in most of the cypselae, except for *L. balansae* (Fig. 1H). Stomata are located in middle region of the fruit of *L. setososquamosa* (Fig. 7A).

Floral disk: The floral disk is in the apical region of the fruit where the pappus is inserted. In the analyzed species, the floral disk is formed of uniseriate exocarp, with periclinally elongated and juxtaposed cells (Fig. 7B, C). The

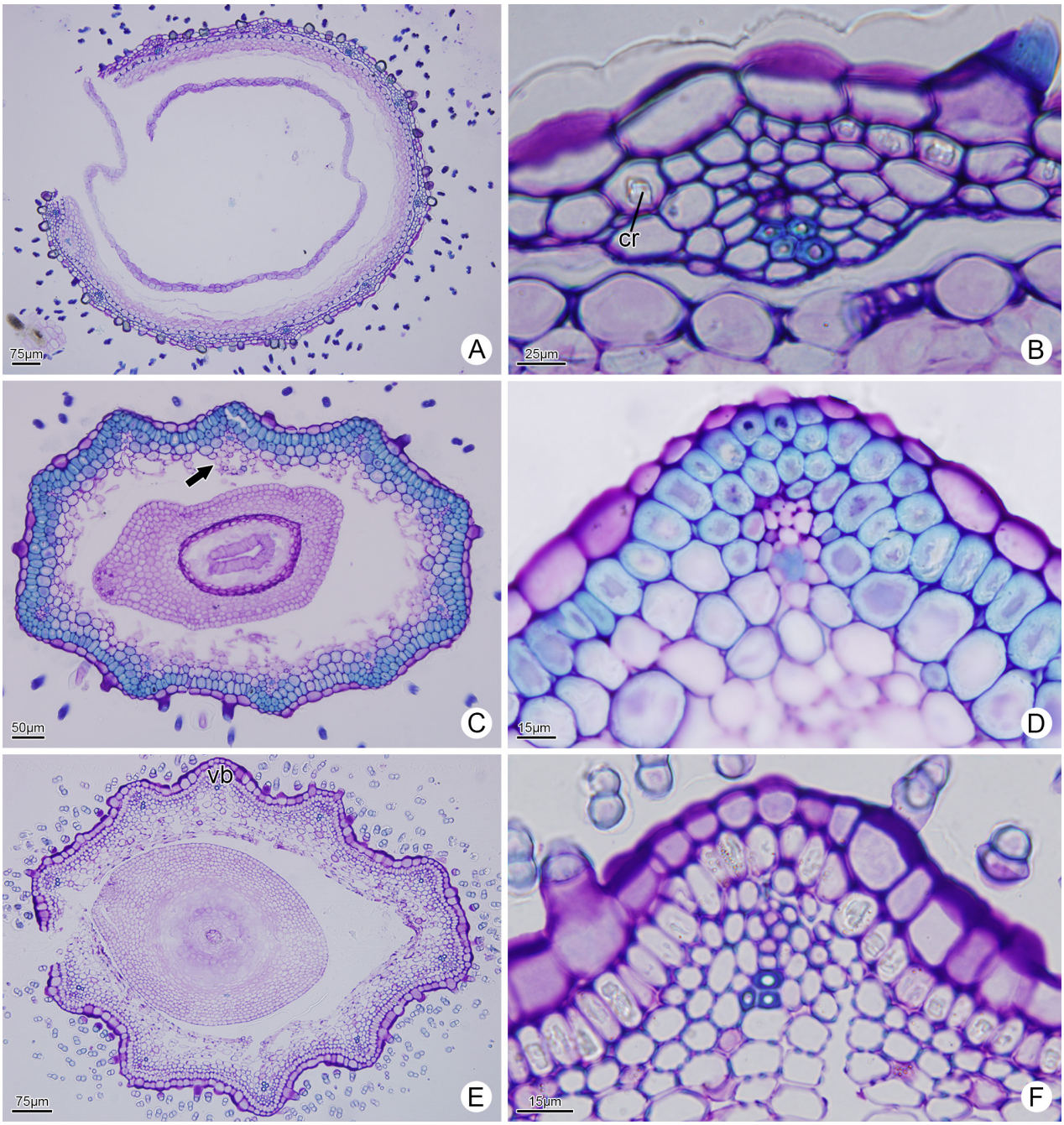

Figure 5. Transversal sections of the fruit of the studied species of *Lepidaploa*: **A-B.** *L. psilostachya*; **C-D.** *L. remotiflora*; **E-F.** *L. salzmannii*; arrow: disintegrating inner mesocarp; cr: crystal; se: seed; vb: vascular bundles.

periphery of the inner region of the floral disk is composed of anticlinally elongated cells, while its center is formed of lignified cells with crystals (Fig. 7B, C).

Pappus: The pappus of all the analyzed species is biseriate (Fig. 7D, E). In the median transverse section, the outer series has a fusiform shape, while the inner series is composed of rounded bristles (Fig. 7D, E). Both series of the pappus are formed by lignified rounded cells with projections and vascularized bristles (Fig. 7D, E).

Carpopodium: In all the species studied, the carpopodium is formed of uniseriate exocarp with periclinally elongated and juxtaposed cells (Fig. 7F, G). The wall of the cells of the exocarp, in most of the species, is lignified (Fig. 7F) (Tab. 2), but in *L. amambaia*, *L. argyrotricha*, *L. buchtienii*, *L. myriocephala*, *L. salzmannii* (Fig. 7G) (Tab. 2), and *L. setososquamosa,* this wall is not lignified. Internally, the carpopodium possesses rounded primary wall cells in the periphery, while in its center there is a vascular bundle.

Seeds: All seeds are immature, except in *L. psilostachya* and *L. tarijensis*. The exotesta of *L. psylostachya* (Fig. 5B) possesses U-shaped thickening, while in *L. tarijensis* (Fig. 6G) lignification occurs only in the anticlinal wall of the

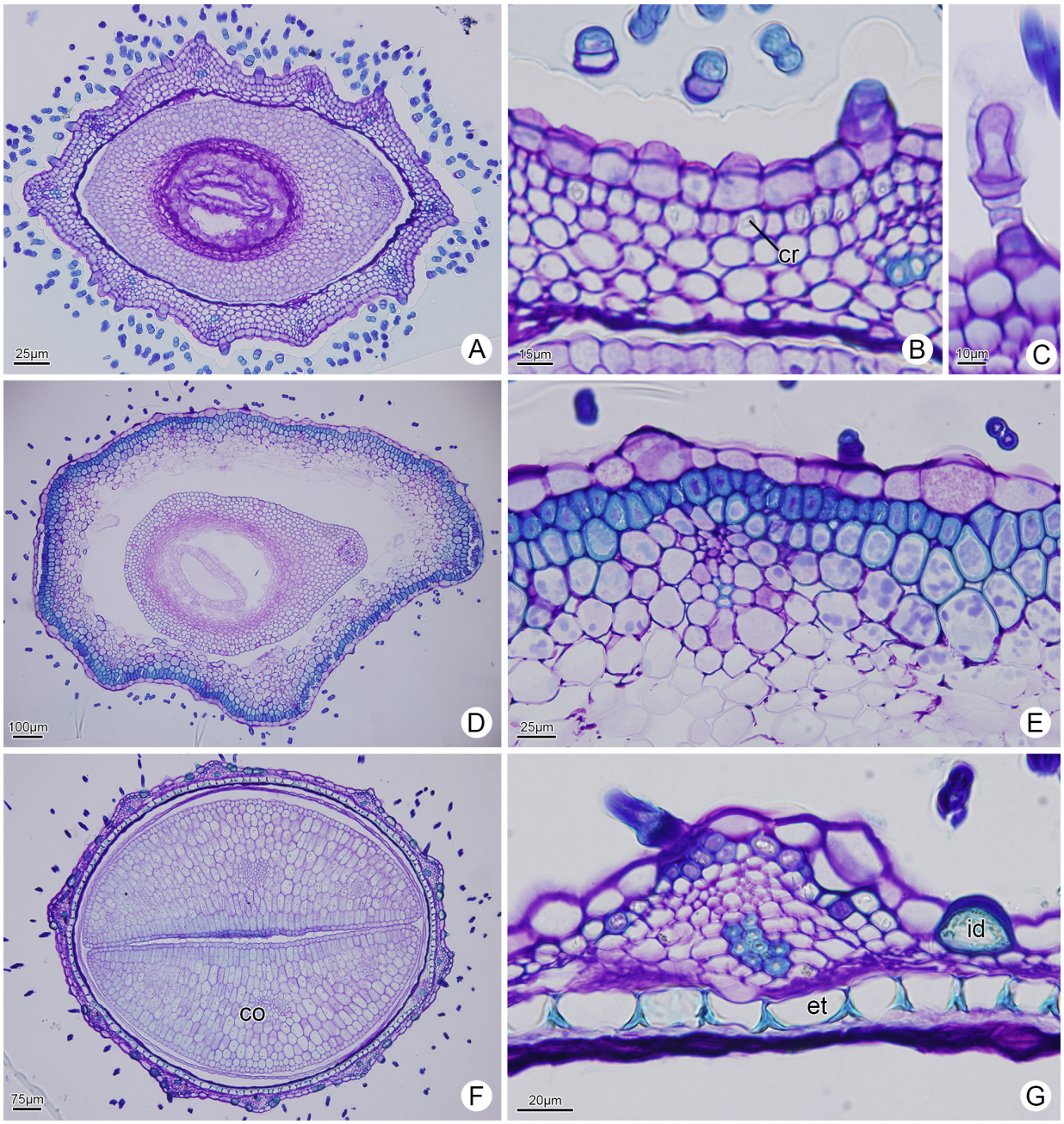

Figure 6. Transversal sections of the fruit of the studied species of *Lepidaploa*: **A-C.** *L. setososquamosa*, in C detail of a glandular trichome; **D-E.** *L. sordidopapposa*; **F-G.** *L. tarijensis*, in G detail of lignified exotesta. co: cotyledon; cr: crystals; et: exotesta; id: idioblast; vb: vascular bundles.

exotesta. All of the species have only one vascular bundle (Figs. 1G; 2G; 3G), which passes through the region of the raphe to the region of the chalaza.

Maturation of cypsela: Differences between the outer mesocarp cells of immature and mature cypselae occur in eight species (*L. canescens*, *L. mapirensis*, *L. myriocephala*, *L. pseudomuricata*, *L. remotiflora*, *L. setososquamosa*, *L. sordidoppaposa*, and *L. tarijensis*). Immature cypselae do not possess lignification in the outer mesocarp (Fig. 7H, I), while in mature cypsela this region possesses cells lignified (Fig. 7H, I).

Discussion

All of the species analyzed of *Lepidaploa* exhibited the same structural pattern of the pericarp that has been

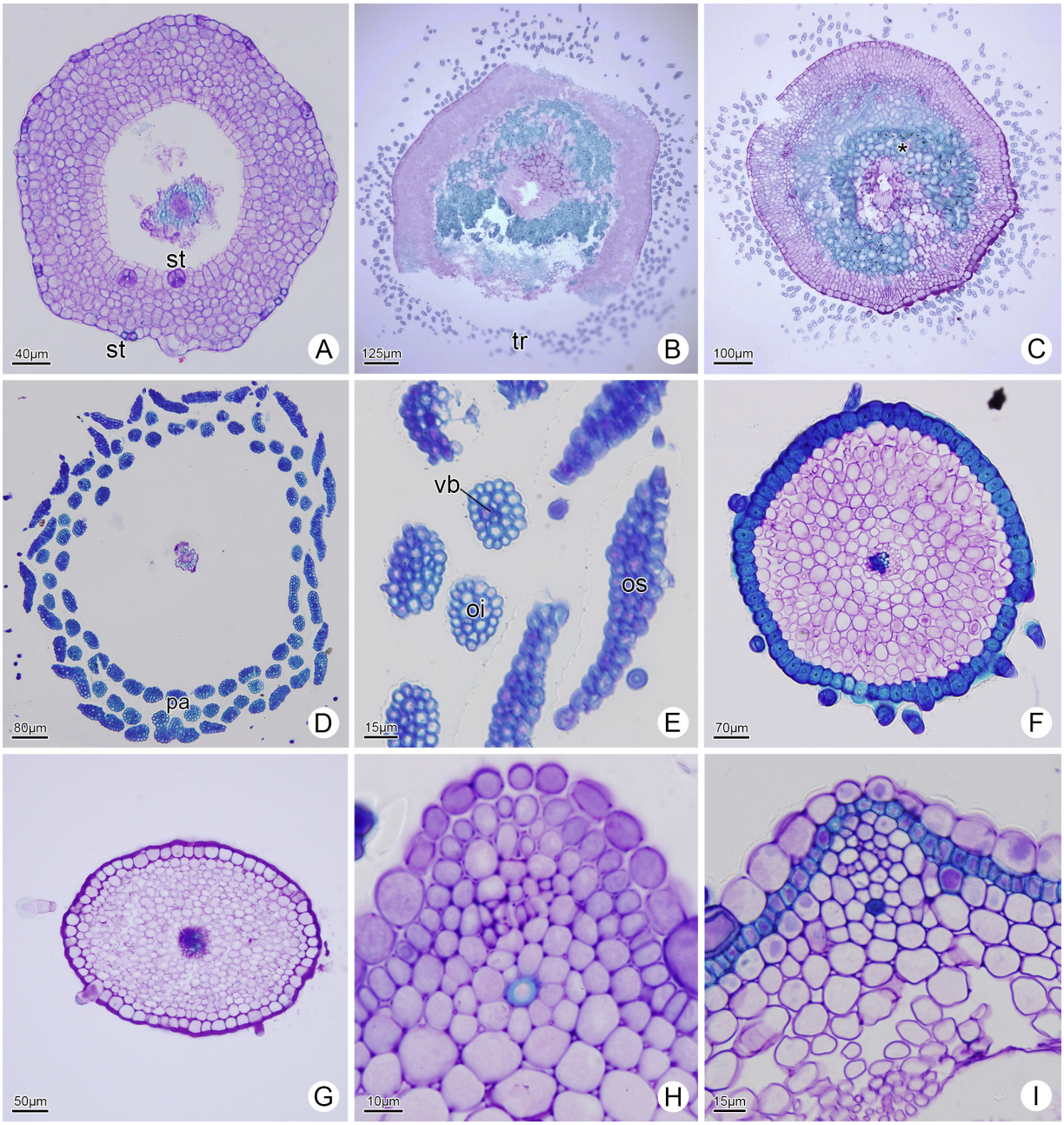

Figure 7. Middle region of cypsela, floral disk, pappus, carpopodium and ribs of the studied species in transversal section. **A.** *L. setososquamosa*, detail of the stomata in the exocarp and endocarp; **B.** *L. eriolepis*, floral disk; **C.** *L. salzmannii*, detail of lignification (asterisk) in the floral disk; **D-F.** *L. amambaia*, outer and inner series of the pappus; F: *L. beckii*, detail of the carpopodium with lignified cells in the exocarp; **G.** *L. salzmannii*, detail of the carpopodium without lignified cells in the exocarp; **H-I.** *L. balansae*, detail comparing non-lignified outer mesocarp and lignified outer mesocarp. oi: inner series of the pappus; os: outer series of the pappus; pa: pappus; st: stomata; tr: trichome; vb: vascular bundles.

reported for other genera of Vernonieae as in *Bolanosa* (Redonda-Martínez *et al.* 2017), *Bothriocline* (Mukherjee & Sarkar 2001), *Chrysolaena* (Martins & Oliveira 2007; Galastri & Oliveira 2010), *Centratherum* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Critoniopsis* (Redonda-Martínez *et al.* 2017), *Cyanthillium* (Pandey & Singh 1980; Redonda-Martínez *et al.* 2017), *Cyrtocymura* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Elephantopus* (Pandey & Singh 1980; Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Gymnanthemum* (Mukherjee & Sarkar 2001), *Harleya* (Redonda-Martínez *et al.* 2017), *Heterocoma* (Freitas *et al.* 2015), *Leiboldia*, *Lepidonia* (Redonda-Martínez *et al.* 2017), *Lessingianthus* (Martins & Oliveira 2007), *Piptocarpha*, *Pseudoelephantopus* (Redonda-Martínez *et al.* 2017), *Rolandra* (Mukherjee & Sarkar 2001), *Spiracantha*, *Stenocephalum*, *Stramentopappus* (Redonda-Martínez *et al.* 2017), *Vernonanthura* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), and *Vernonia s.s.* (Misra 1972; Pandey & Singh 1980; Mukherjee & Sarkar 2001; Jana & Mukherjee 2013; Redonda-Martínez *et al.* 2017). In other words, the cypselae of *Lepidaploa* possess four different regions, which were generally disintegrated: exocarp, outer mesocarp, inner mesocarp and endocarp.

The uniseriate exocarp also exhibited the same pattern as that found for other Vernonieae genera as in *Bolanosa* (Redonda-Martínez *et al.* 2017), *Bothriocline* (Mukherjee & Sarkar 2001), *Chrysolaena* (Martins & Oliveira, 2007; Galastri & Oliveira 2010), *Centratherum* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Critoniopsis* (Redonda-Martínez *et al.* 2017), *Cyanthillium* (Pandey & Singh 1980; Redonda-Martínez *et al.* 2017), *Cyrtocymura* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Elephantopus* (Pandey & Singh 1980; Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Gymnanthemum* (Mukherjee & Sarkar 2001), *Harleya* (Redonda-Martínez *et al.* 2017), *Heterocoma* (Freitas *et al.* 2015), *Leiboldia*, *Lepidonia* (Redonda-Martínez *et al.* 2017), *Lessingianthus* (Martins & Oliveira 2007), *Piptocarpha*, *Pseudoelephantopus* (Redonda-Martínez *et al.* 2017), *Rolandra* (Mukherjee & Sarkar 2001), *Spiracantha*, *Stenocephalum*, *Stramentopappus* (Redonda-Martínez *et al.* 2017), *Vernonanthura* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), and *Vernonia s.s.* (Misra 1972; Pandey & Singh 1980; Mukherjee & Sarkar 2001; Jana & Mukherjee 2013; Redonda-Martínez *et al.* 2017). However, different sizes for the epidermal cells in the ribs and inter-ribs is reported for the first time. The presence of idioblasts and glandular trichomes in some species is important for taxonomic differentiation, whereas biseriate non-glandular trichomes, which were found in all species, have no taxonomic value within Vernonieae. According to Isawumi *et al.* (1966), idioblasts are generally present in species of Vernonieae and they may be characteristic structures of the tribe. Previous studies report the presence of idioblasts in Asian and African species of Vernonieae (Mukherjee & Sarkar 2001; Basak & Mukherjee 2003; Jana & Mukherjee 2012; Mukherjee & Nordenstam 2012), and American species of the tribe (Angulo *et al.* 2015; Redonda-Martínez *et al.* 2017; Pico *et al.* 2016). Despite their taxonomic importance at the tribal level, idioblasts must be used with care in the delimitation of

Table 2. Morphological and anatomical features of the fruit of the studied species of *Lepidaploa*. ? = unknown.

Species	Pericarp	Exocarp				Mesocarp			Endocarp
	All ribs defined	Glandular trichome	Stomata	Idioblast	Carpopodium	Outer mesocarp: number of layers	Crystals	Number of vascular bundles	Stomata
L. amambaia	no	absent	absent	absent	non-lignified	2	present	10-11	?
L. argyrotricha	yes	absent	absent	present	non-lignified	2	present	10-11	?
L .bakerana	yes	absent	absent	present	lignified	1-2	present	10	?
L. balansae	no	absent	present	present	lignified	1-2	present	10	present
L. beckii	no	absent	absent	present	lignified	1-2	present	10-11	?
L. buchtienii	no	absent	absent	present	non-lignified	1	present	10	?
L. canescens	no	absent	absent	absent	lignified	1-2	present	10	?
L. chamissonis	no	present	absent	present	lignified	1-3	present	10	?
L. costata	yes	absent	absent	present	lignified	1-2	present	10	?
L. deflexa	no	absent	absent	present	lignified	2	present	10	?
L. eriolepis	no	absent	absent	absent	lignified	2	present	10	?
L. mapirensis	no	absent	absent	absent	lignified	1-2	present	10	?
L. myriocephala	no	absent	absent	absent	non-lignified	2-4	present	10	?
L. novarae	yes	absent	absent	present	lignified	1	present	10	?
L. pseudomuricata	yes	present	absent	present	lignified	1-2	present	10	?
L. psilostachya	no	absent	absent	present	lignified	2	present	10	?
L. remotiflora	no	absent	absent	present	lignified	1-3	present	10	?
L. salzmannii	yes	present	absent	present	non-lignified	1-2	present	10	?
L. setososquamosa	no	present	present	present	non-lignified	1-2	present	10	present
L. sordidopapposa	no	absent	absent	present	lignified	1-2	present	10	?
L. tarijensis	yes	absent	absent	present	lignified	1-2	present	10	?

species. The presence of a glandular trichome in four species is an important taxonomic character for the differentiation of some entities. For example, among the closely related species *L. amambaia*, *L. remotiflora* and *L. setososquamosa*, the last can be differentiated because it is the only one of this group that possesses glandular trichomes on the cypsela. Another species that also possesses this type of trichome is *L. chamissonis*, differentiating it from *L. argyrotrichia,* which does not possess glandular trichomes in the fruit.

The outer mesocarp exhibited two patterns: layers of cells non lignified or layers of cells lignified. This variation may be due to different stages of fruit development since we analyzed immature and mature cypselae of eight species (*L. canescens*, *L. mapirensis*, *L. myriocephala*, *L. pseudomuricata*, *L. remotiflora*, *L. setososquamosa*, *L. sordidoppaposa* and *L. tarijensis*). Ontogenic studies of the fruit of *Chrysolaena obovata* (Galastri & Oliveira 2010) reported four stages of maturation and demonstrated the structural differences of the outer mesocarp in these stages. Galastri & Oliveira (2010) observed that the outer and inner mesocarp differs in shape and size, and that there is gradual lignification. We also observed the pattern found in *Chrysolaena obovata* in the eight species of *Lepidaploa* mentioned above. Crystals were observed only in the outer mesocarp of all species. In transversal section, these crystals have a quadratic or rectangular shape. The presence of crystals is common within Vernonieae (Misra 1972; Pandey & Singh 1980; Mukherjee & Sarkar 2001; Martins & Oliveira 2007; Galastri & Oliveira 2010; Redonda-Martínez *et al.* 2017). According to King & Robinson (1987), the production of crystals in fruits is possibly antagonistic to the production of phytomelanin, a black substance found in the cypselae of species of Eupatorieae (Robinson *et al*. 2009; De-Paula *et al.* 2013), Heliantheae (Baldwin 2009), and *Heterocoma* (Freitas *et al.* 2015) (Vernonieae). Considering the antagonism hypothesis of King & Robinson (1987), we did not find phytomelanin in the cypselae of the species of *Lepidaploa* studied here.

The outer mesocarp of all *Lepidaploa* studied is continuous, and the number of layers present in this region does not exhibit great interspecific variability. Therefore, this region does not have taxonomic value among species of *Lepidaploa,* as demonstrated for the genera and species studied by Redonda-Martínez *et al.* (2017). In relation to the taxonomic importance of the outer mesocarp at the generic level, *Chrysolaena* (as seen in *C. obovata* and *C. platensis*) possesses the same number of cell layers (Martins & Oliveira 2007; Galastri & Oliveira 2010) as its sister genus, *Lepidaploa*. Meanwhile, *Lessingianthus brevifolius* (Martins & Oliveira 2007), a species of the sister genus of *Chrysolaena* and *Lepidaploa*, exhibited only one layer of cells in the outer mesocarp, and so may represent a taxonomic difference between *Chrysolaena* + *Lepidaploa* and *Lessingianthus*, if this same pattern is confirmed in the other species of *Lessingianthus*. Although this character is sufficient to differentiate *Lessingianthus brevifolius* from the other two genera, the number of cell layers must be used with care in the taxonomy of the group, since there are records in the literature of variation in the number of layers in different regions of the same fruit, as reported for *Cyanthillium cinereum* (Tiagi & Taimni 1960; Pandey & Singh 1980), *V. fasciculata, V. missurica* (Pandey & Singh 1980), and *V. anthelmintica* (Misra 1972; Pandey & Singh 1980; Talukdar 2015).

Variation in the number of ribs compared to the number of vascular bundles of the cypselae was found among the species analyzed. The number of vascular bundles does not necessarily correspond to the number of ribs since there are ribs in the fruits of *Lepidaploa* that are undefined. Differences between the number of ribs and the number of vascular bundles were also found for *Vernonia bainesii*, *V. cistifolia* (Mukherjee & Sarkar 2001), *V. galamensis* (Jana & Mukherjee 2013), *Cyanthillium cinereum* (Pandey & Singh 1980), *Chrysolaena obovata*, *Lessingianthus brevifolius* (Martins & Oliveira 2007), and *Chrysolaena platensis* (Galastri & Oliveira 2010). According to Marzinek *et al.* (2010), the formation of ribs is related to the position occupied by the flower in the capitula, which would generate the variability in the ribs of *Lepidaploa*. Our anatomical studies show that the number of ribs must be used carefully in the delimitation of genera and species, because several taxonomic studies have used the number of ribs to separate species, and sometimes genera, within the Vernonieae. However, in *Lepidaploa* the number of ribs is not taxonomically informative, given that some ribs are not conspicuous and cannot be seen with external morphological study.

The shape of the cells and the type of cell wall of the inner mesocarp found in the present study is similar that found in other Vernonieae genera, such as *Bolanosa* (Redonda-Martínez *et al.* 2017), *Bothriocline* (Mukherjee & Sarkar 2001), *Chrysolaena* (Martins & Oliveira, 2007; Galastri & Oliveira 2010), *Centratherum* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Critoniopsis* (Redonda-Martínez *et al.* 2017), *Cyanthillium* (Pandey & Singh 1980; Redonda-Martínez *et al.* 2017), *Cyrtocymura* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Elephantopus* (Pandey & Singh 1980; Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), *Gymnanthemum* (Mukherjee & Sarkar 2001), *Harleya* (Redonda-Martínez *et al.* 2017), *Heterocoma* (Freitas *et al.* 2015), *Leiboldia*, *Lepidonia* (Redonda-Martínez *et al.* 2017), *Lessingianthus* (Martins & Oliveira 2007), *Piptocarpha*, *Pseudoelephantopus* (Redonda-Martínez *et al.* 2017), *Rolandra* (Mukherjee & Sarkar 2001), *Spiracantha*, *Stenocephalum*, *Stramentopappus* (Redonda-Martínez *et al.* 2017), *Vernonanthura* (Mukherjee & Sarkar 2001; Redonda-Martínez *et al.* 2017), and *Vernonia s.s.* (Misra 1972; Pandey & Singh 1980; Mukherjee & Sarkar 2001; Jana & Mukherjee 2013; Redonda-Martínez *et al.* 2017). It is worth pointing out that the pericarp of species of Vernonieae is non-multiplicative, which is confirmed by

the consumed inner mesocarp of the cypselae of, mainly, *L. psylostachya* and *L. tarijensis*. According to Martins & Oliveira (2007), the small size of the pericarp of *Chrysolaena obovata* and *Lessingianthus brevifolius* favors dispersion by anemochory because the reduced number of layers of this region makes the fruit lighter. The consumed of the inner mesocarp during fruit maturation was also observed for the species studied by Tiagi & Taimni (1960) and Misra (1972), and in recent works with *Chrysolaena obovata* and *Lessingianthus brevifolius* (Martins & Oliveira 2007) and *Chrysolaena platensis* (Galastri & Oliveira 2010).

The floral disk is similar to the some species of *Vernonia* s.s. (Pandey & Singh 1980), and in *Chrysolaena platensis* (Galastri & Oliveira 2010). There were no significant differences among the floral disks of the different species analyzed in the present study, with the exception that some possessed lignified exocarp lignified in this region. However, this difference in lignin deposition in the exocarp wall may be related to the degree of maturity of the fruit, as suggested for the region of the outer mesocarp. Galastri & Oliveira (2010) observed crystals on floral disks in *Chrysolaena platensis*, but Marzinek & Oliveira (2010) and Silva *et al.* (2018) described only phytomelanin in this structure of species of Eupatorieae.

The basal region known as carpopodium is present in all the species analyzed here. The carpopodium anatomy was previously studied only for *Chrysolaena obovata*, *Lessingianthus brevifolius* (Martins & Oliveira 2007) and *Chrysolaena platensis* (Galastri & Oliveira 2010). The carpopodium of *Lepidaploa* is similar to the carpopodium found in *Chrysolaena* (Martins & Oliveira 2007) and *Lessingianthus* (Galastri & Oliveira 2010). In other words, this structure presents a uniseriate exocarp and its interior has parenchyma cells containing a group of sclereids in the center. Among the anatomical features studied, lignification in the exocarp of the carpopodium is the one that proved most useful for differentiating species. Some species exhibited an exocarp with a non-lignified wall, while the exocarp of other taxa possessed lignification of this region. We observed this difference among cypselae at the same stage of maturation, which indicates that it can be a useful character for differentiating closely related species. For example, *L. remotiflora*, a close relative of *L. amambaia* and *L. setososquamosa*, is the only one that possesses lignified cells of the exocarp of the carpopodium, while the others lack lignification. This difference is also important for separating *L. argyrotricha* (carpopodium with non-lignified exocarp) from *L. chamissonis* (carpopodium with lignified exocarp).

The seeds of *Lepidaploa* provide important taxonomic data, especially the characters of the testa. The exotesta of the seeds of *L. psylostachya* and *L. tarijensis* possess a secondary wall in the anticlinal and periclinal walls, and only in the anticlinal wall, respectively. The presence of a secondary wall of the exotesta was also reported in *Vernonia anthelmintica*, which exhibited U-shaped lignification (Talukdar 2015). It is worth mentioning that Jana & Mukherjee (2013) did not observe the presence of a secondary wall in the exotesta of the same specimen of *V. anthelmintica,* which could be related to the degree of maturity of the cypsela. In *Chrysolaena platensis* (Galastri & Oliveira 2010), the region of the mesotesta and endotesta are consumed in the mature seed, however, lignin deposition was observed in the stages prior to the collapse of this structure.

The pappus is an important modification of the sepals that acts in the dispersion of the fruit (Roth 1977). We observed lignified cells with projections in the bristles of *Lepidaploa*; both structures were also found in species of Eupatorieae (Marzinek & Oliveira 2010) and in *Chrysolaena obovata* and *Lessingianthus brevifolius* (Martins & Oliveira 2007) of Vernonieae. Similarly, the presence of vascular bundles in the interior of each bristle of *Lepidaploa* was also found in other genera of Eupatorieae (King & Robinson 1987; Marzinek & Oliveira 2010) and Vernonieae (Misra 1972). However, previous studies show that vascular bundles are absent in the bristles of *Chrysolaena obovata*, *Lessingianthus brevifolius* (Martins & Oliveira 2007) and *Chrysolaena platensis* (Galastri & Oliveira 2010). Although vascular bundles are absent in species of *Chrysolaena* and *Lessingianthus*, more species of these genera need to be studied in order to establish the taxonomic validity of this absence. According to Mukherjee & Sarkar (2001), the pappus is an important structure for the identification of genera and species. The species of *Lepidaploa* exhibited the same pattern for the pappus, and so the anatomy of this structure cannot be used as a taxonomic tool to separate these entities. On the other hand, the anatomy of the pappus could be important for distinguishing *Lepidaploa* from *Chrysolaena* and *Lessingianthus*. In *Chrysolaena* (Martins & Oliveira 2007; Galastri & Oliveira 2010) and *Lessingianthus* (Martins & Oliveira 2007), the bristles of the pappus do not possess vascular bundles, which would differentiate these two genera from the closely related genus *Lepidaploa*, which possesses vascular bundles in the pappus.

Conclusion

The cypselae of *Lepidaploa* species exhibited biseriate trichomes, uniseriate exocarp, outer mesocarp (generally with one or two layers of cells and the presence of crystals), and vascular bundles in the inner mesocarp; this morphology is similar to that of other taxa of Vernonieae. However, there are important taxonomic differences between species in relation to idioblasts, glandular trichomes, lignification of the carpopodium, and pappus vascularization. The significance of carpopodium lignification for identifying species of *Lepidaploa* is reported here for the first time. In addition, the pappus vascularization and number of mesocarp layers could provide important taxonomic data for segregating *Chrysolaena*, *Lepidaploa* and *Lessingianthus* genera.

Acknowledgements

We thank Consejo Nacional de Investigaciones Científicas y Técnicas and Agencia Nacional de Promoción Científica y Tecnológica for providing financial support.

References

Anderson LC. 1963. Studies on *Petradoria* (Compositae): anatomy, cytology, taxonomy. Transactions of the Kansas Academy of Science 66: 632-684.

Angulo MB, Sosa MM, Dematteis M. 2015. Systematic significance of cypsela morphology in *Lessingianthus* (Vernonieae, Asteraceae). Australian Systematic Botany 28: 173-189.

Baldwin BG 2009. Heliantheae alliance. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ. (eds.) Systematics, evolution and biogeography of Compositae. Vienna, IAPT. p. 689-711.

Basak N, Mukherjee SK 2003. Taxonomic significance of cypselar features in some species of *Vernonia* (Vernonieae-Asteraceae). Journal of Hill Research 1: 9-15.

De-Paula OC, Marzinek J, Oliveira DMT, Machado SRM. 2013. The role of fibers and the hypodermis in Compositae melanin secretion. Micron 44: 312-316.

Freitas FS, De-Paula OC, Nakajima JN, Marzinek J. 2015. Fruits of *Heterocoma* (Vernonieae-Lychnophorinae): taxonomic significance and a new pattern of phytomelanin deposition in Asteraceae. Botanical Journal of the Linnean Society 179: 255-265.

Funk VA, Bayer RJ, Keeley S, *et al*. 2005. Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. Biologiske Skrifter 55: 343-374.

Galastri N, Oliveira DMT. 2010. Morfoanatomia e ontogênese do fruto e semente de *Vernonia platensis* (Spreng.) Less. (Asteraceae). Acta Botanica Brasilica 24: 73-83.

Isawumi MA, El-Ghazaly G, Nordenstam B. 1996. Pollen morphology, floral microcharacters and taxonomy of the genus *Baccharoides* Moench (Vernonieae: Asteraceae). Grana 35: 205-230.

Jana BK, Mukherjee SK. 2012. Variations of cipselas of five taxa of the tribe Vernonieae (Asteraceae). International Journal of Pharma and Bio Sciences 1: 420-435.

Jana BK, Mukherjee SK. 2013. Cypselar anatomy of some taxa of Compositae. Journal of Chemical and Pharmaceutical Research 2: 417-443.

Keeley SC, Forsman ZH, Chan R. 2007. A phylogeny of the "evil tribe" (Vernonieae: Compositae) reveals Old/New World long distance dispersal: Support from separate and combined congruent datasets (trnL-F, ndhF, ITS). Molecular Phylogenetics and Evolution 44: 89-03.

Keeley SC, Jansen RK. 1994. Chloroplast DNA restriction site variation in the Vernonieae (Asteraceae), an initial appraisal of the relationship of New and Old World taxa and the monophyly of *Vernonia*. Plant Systematics and Evolution 193: 249-265.

Keeley SC, Robinson H. 2009. Vernonieae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ. (eds.) Systematics, evolution and biogeography of Compositae. Vienna, IAPT p. 439-469.

King RM, Robinson H. 1987. The genera of the Eupatorieae (Asteraceae). Monographs in Systematic Botany 22. St. Louis, Missouri Botanical Garden.

Marques D, Dematteis M. 2014. *Lepidaploa* (Cass.) Cass. In: Zuloaga FO, Belgrano MJ, Anton AM. (eds.) Flora Argentina, flora vascular de la República Argentina Dicotyledoneae Asteraceae: *Senecio* a Vernonieae. Buenos Aires, IBODA CONICET. p. 243-249.

Martins MAG, Oliveira DMT. 2007. Morfoanatomia comparada dos frutos em desenvolvimento de *Vernonia brevifolia* Less. e *V. herbacea* (Vell.) Rusby (Asteraceae). Revista Brasileira de Botânica 30: 101-112.

Marzinek J, De-Paula OC, Oliveira DMT. 2010. The ribs of Eupatorieae (Asteraceae): of wide taxonomic value or reliable characters only among certain groups? Plant Systematics and Evolution 285: 127-130.

Marzinek J, Oliveira DMT. 2010. Structure and ontogeny of the pericarp of six Eupatorieae (Asteraceae) with ecological and taxonomic considerations. Anais da Academia Brasileira de Ciências 82: 279-291.

Misra BS. 1972. Floral Morphology of the family Compositae V. The seed coat and pericarp in *Verbesina encelioides* (Cav.) Benth.and Hk. F. ex. A Gray. Journal of the Indian Botanical Society 51: 332-341.

Mukherjee SK, Nordenstam B. 2012. Diversity of trichomes from mature cypselar surface of some taxa from basal tribes of Compositae. Compositae Newslett 50:78-125

Mukherjee SK, Sarkar AK. 2001. Study of macro-morphological and anatomical structures of cypselas of eighteen taxa of the tribe Vernonieae (Asteraceae). Journal of the National Botanical Society India 55: 85-104.

O'Brien TP, Feder N, McCully ME. 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59: 368-373.

Pandey AK, Singh RP. 1980. Development and Structure of Seeds and Fruits in Tribe Vernonieae - some *Vernonia* and *Elephantopus* species. Flora 169: 443-452.

Pico GMV, Veja AJ, Dematteis M. 2016. Systematic consideration of floral microcharacters of the South American genus *Chrysolaena* (Vernonieae, Asteraceae). Systematics and Biodiversity 14: 224-243.

Pruski JF. 2017. Compositae of Central America-VIII. The genus *Lepidaploa* (Vernonieae). Phytoneuron 50: 1-39.

Redonda-Martínez R, Terrazas T, Rojas-Leal A, Villaseñor JL. 2017. The morpho-anatomy of cypselae in subtribe Leiboldiinae (Vernonieae, Asteraceae). Brazilian Journal of Botany 40: 491-502.

Redonda-Martínez R, Villaseñor JL. 2011. El género *Lepidaploa* (Familia Asteraceae, Tribu Vernonieae) en México. Revista Mexicana de Biodiversidad 82: 782-797.

Robinson H. 1999. Generic and subtribal classification of American Vernonieae. Smithsonian Contributions to Botany 89: 1-116.

Robinson H, Schilling E, Panero JE. 2009. Eupatorieae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ. (eds.) Systematics, evolution and biogeography of Compositae. Vienna, IAPT. p. 731-744.

Roth I. 1977. Fruits of angiosperms. Handbuch der Pflanzenanatomie. Berlin, Stuttgart.

Semir J, Rezende AR, Monge M, Lopes NP. 2011. As Arnicas endêmicas das Serras do Brasil: uma visão sobre a biologia e a química das espécies de *Lychnophora* (Asteraceae). Ouro Preto, UFOP.

Silva TDG, Marzinek J, Hattori EKO, Nakajima JN, De-Paula OC. 2018. Comparative cypsela morphology in Disynaphiinae and implications for their systematics and evolution (Eupatorieae: Asteraceae). Botanical Journal of the Linnean Society 186: 89-107.

Talukdar T. 2015. Taxonomic significance of cypsela in *Vernonia anthelmintica* Willd. and *V. cinerea* Less. (Asteraceae; tribe - Vernonieae): Structural manifestations. Plant Science Today 2: 107-115.

Tiagi B, Taimni S. 1960. Embryo-sac development in *Vernonia cinerascens* Schult. and seed development in *V. cinerea* Less. Current Science 30: 406.